CLAIMS

1. A method for detecting an interaction between a first membrane bound protein or part thereof and a second protein or part thereof which is either membrane bound or soluble, the method comprising:

(a) providing a host cell containing at least one detectable gene (reporter gene) having a binding site for a transcriptional activator, such that the detectable gene expresses a detectable product, preferably a protein, when the detectable gene is transcriptionally activated;

(b) providing, as part of a bait vector, a first chimeric gene capable of being expressed in said host cell, the first chimeric gene coding *inter alia* for a first membrane protein or part thereof which gene is attached to the DNA-sequence of a first module encoding *inter alia* a first protein sequence involved in intracellular protein degradation and a transcriptional activator, said first protein or part thereof to be tested whether it can interact with a second protein or part thereof;

(c) providing, as part of a prey vector, a second chimeric gene capable of being expressed in said host cell, the second chimeric gene coding *inter alia* for a second protein or part thereof which is either membrane bound or soluble and which gene is attached to the DNA sequence of a second module encoding *inter alia* a second protein sequence involved in intracellular protein in degradation;

(d) introducing the bait vector and the prey vector into the host cell such that an interaction between the expressed first and second protein and/or their parts can take place, which interaction leads to an interaction of the first protein sequence of the first module and the second protein sequence of the sec-

ond module which interaction in turn leads to activation of an intracellular protease and proteolytic separation of the transcriptional activator, at least one of the bait vector and prey vector, preferably both, being suitable for being maintained episomally;

(e) determining whether the detectable gene of the host cell has been activated by the transcriptional activator.

2. The method according to claim 1, wherein the host cell is a yeast, a bacterial or a mammalian cell.

3. The method according to claim 2, wherein the host cell is *a Saccharomyces pombe* cell or, more preferably, cells of the budding yeast *Saccharomyces cerevisiae*.

4. The method according to any of claims 1 to 3, wherein the detectable gene is activated by a natural or artificial activator, preferably an activator comprising a short tagging module.

5. The method according to any of claims 1 to 4, wherein the detectable gene is activated by the artificial transcriptional activator protein A-LexA-V16 (PLV).

6. The method according to any of claims 1 to 5, wherein the first protein sequence comprises a C-terminal portion of ubiquitin (Cub) or a mutant thereof (CbM) and the second protein sequence comprises an N-terminal portion of ubiquitin (Nub) or a mutant thereof (NbM).

7. The method according to any of claims 1 to 6, wherein the DNA-sequence coding for the first membrane protein is selected from the group consisting of any bacterial membrane protein, any viral membrane protein, any oncogene-encoded membrane protein, any growth factor receptor or any eukaryotic membrane protein, or parts thereof.

8. The method according to any one of the preceding claims wherein the second membrane protein or the soluble protein, or part thereof, is encoded by a library of plasmids.

9. A kit for detecting binding between a first membrane bound protein or part thereof and a second protein or part thereof which is either membrane bound or soluble comprising:

(a) a host cell containing at least one detectable gene (reporter gene) having a binding site for a transcriptional activator, such that the detectable gene expresses a detectable product, preferably a protein, when the detectable gene is transcriptionally activated;

(b) a first vector (bait) comprising a first site capable of receiving a first nucleic acid coding for a first membrane protein or part thereof such that when the first nucleic acid is inserted it becomes attached to the DNA sequence of a first module encoding *inter alia* a first protein sequence involved in intracellular protein degradation, the module further comprising a nucleic for a transcriptional activator;

(d) a second vector (prey) comprising a second site capable of receiving a second nucleic acid coding for a second membrane protein or a soluble protein or part thereof such that when the second nucleic acid is inserted it becomes attached to the DNA sequence of a second module encoding inter alia a second protein sequence involved in intracellular protein degradation; and optionally

(e) a plasmid library encoding second proteins or parts thereof.

10. The kit according to claim 9, wherein the host cell is a yeast, bacterial or mammalian cell.

11. The kit according to claim 10, wherein the host cell is a yeast cell, preferably of *Saccharomyces pombe* and most preferably of the budding yeast *Saccharomyces cerevisiae.*

12. The kit according to any of claims 9 to 11, wherein the detectable gene can be activated by a natural or artificial activator, preferably an activator comprising a short tagging module.

13. The kit according to any of claims 9 to 12, wherein the detectable gene can be activated by the artificial transcriptional activator Protein A-LexA-V16 (PLV).

14. The kit according to any of claims 9 to 13, wherein the first protein sequence contains the C-terminal part of ubiquitin (Cub) or a mutant thereof and the second protein sequence contains the N-Terminal part of ubiquitin (Nub) or a mutant thereof.

15. The kit according to any of claims 9 to 14, wherein the promoter is selected from the group consisting of ADH promoter, CYC1 promoter or TEF1 promoter.

16. The kit according to any of claims 9 to 15, wherein the DNA-sequence coding for the first membrane protein is derived from any bacterial membrane protein, any viral membrane protein, any oncogene-encoded membrane protein, any growth factor receptor or any eukaryotic membrane protein, or parts thereof.

17. The kit according to any of claims 9 to 16, wherein the DNA-sequence coding for the second protein is contained in a library of plasmids.

18. Vector useful as a bait vector in a method according to any of claims 1 to 8 or a kit according to any of claims 9 to 17 comprising the following elements:

(1) a selection marker for propagation of the vector in *E. coli*;
(2) an origin of replication which allows propagation of the vector in *E. coli*;

(3) a further selection marker for propagation of the vector in yeast;

(4) an origin of replication which allows propagation of the vector in yeast; and

(5) an expression cassette comprising the following elements:

(a) a promoter element conferring low level expression or inducible expression or high level expression;

(b) a nucleic acid sequence encoding a leader;

(c) a site capable of receiving a nucleic acid encoding a protein;

(d) a nucleic acid sequence encoding the C-terminal part of yeast ubiquitin (Cub) or mutated yeast ubiquitin (CbM);

(e) a nucleic acid sequence encoding a tag that allows detection of the fusion protein;

(f) a nucleic acid sequence encoding a DNA binding protein;

(g) a nucleic acid sequence encoding a transcriptional activator; and

(h) a terminator sequence.

19. The vector according to claim 18, wherein the selection marker according to element (1) comprises a marker selected from the ampicilline resistance gene, the kanamycine resistance gene or the chloramphenicol resistance gene.

20. The vector according to any of claims 18 or 19, wherein the origin of replication according to element (2) is selected from a pUC based or pBR322 origin of replication.

21. The vector according to any of claims 18 to 20, wherein the selection marker according to element (3) is selected from any marker that is selectable in *S. cerevisiae*, preferably the LEU2 gene.

22. The vector according to any of claims 18 to 21, wherein the origin of replication according to element (4) is a low copy number origin, preferably selected from CEN and ARS origins.

23. The vector according to any of claims 18 to 22, wherein the promoter according to element (5a) is selected from CYC1, GAL1, CUP1, ADH1 or TEF1.

24. The vector according to any of claims 18 to 23, wherein the nucleic acid sequence encoding a leader according to element (5b) is selected from a signal sequence derived from a yeast integral membrane protein or a signal sequence which confers fatty acid modification preferably to the following peptide (N-MGCTLSAEDKPGGP-C).

25. A vector according to any of claims 18 to 24, wherein the site for receiving a nucleic acid sequence according to feature (5c) is a multiple cloning site or a sequence allowing the insertion of a further nucleic acid sequence by *in vivo* recombination.

26. A vector according to any of claims 18 to 25, wherein the nucleic acid sequence according to element (5d) encodes the C-terminal part of yeast ubiquitin from amino acids 35 to 76.

27. A vector according to any of claims 18 to 26, wherein the tag for detecting the expression of the polypeptide fusion is selected from epitopes of Gal4, LexA, B42 or VP16 or 3x FLAG, 3x MYC or HA epitopes or the hemagglutinin epitopes.

28. A vector according to any of claims 18 to 27, wherein the nucleic acid encoding the DNA binding protein according to element (5f) is selected from the sequence encoding the bacterial LexA protein, the sequence encoding the yeast Gal4 protein, more preferably amino acids 1 to 93, most preferably the sequence encoding amino acids 1 to 74 of the Gal4 protein.

29. A vector according to any of claims 18 to 28, wherein the transcriptional activator according to element (5g) is selected from the *Herpes simplex* virus protein VP16 or more preferably the acidic domain B42.

30. A vector according to any of claims 18 to 29, wherein the terminator sequence according to element (5h) is selected from the CYC1 or ADH1 terminator sequence.

31. A vector according to any of claims 18 to 30, wherein the vector further contains a sequence coding for a first membrane bound protein or part thereof (the bait protein).

32. A vector suitable as a prey vector in a method according to any of claims 1 to 8 or a kit according to any of claims 9 to 17 comprising the following elements:

(1) a selection marker for propagation of the vector in *E. coli*;
(2) an origin of replication which allows propagation of the vector in *E. coli*;
(3) a selection marker for propagation of the vector in yeast;
(4) an origin of replication which allows propagation of the vector in yeast; and
(5) an expression cassette comprising the following elements:
    (a) a promoter element conferring low level expression or inducible expression or high level expression;
    (b) an open reading frame encoding the N-terminal part of yeast ubiquitin (Nub) or a mutated ubiquitin (NbM);
    (c) a nucleic acid sequence encoding a tag which allows detection of the expressed polypeptide fusion;
    (d) a site capable of receiving a nucleic acid sequence encoding a protein; and
    (e) a terminator sequence.

33. The vector according to claim 32, wherein the selection marker according to element (1) is selected from the ampicilline resistance gene, the kanamycine resistance gene and the chloramphenicol resistance gene.

34. The vector according to any of claims 32 or 33, wherein the origin of replication according to element (2) is selected from an origin such as pUC based or

pBR322 based origin or preferably an origin of replication allowing a high copy number of the plasmid when propagated in *E. coli*.

35. The vector according to any of claims 32 to 34, wherein the selection marker according to element (3) is selected from a marker that is selectable in *S. cerevisiae*, preferably a sequence encoding the TRP1 gene.

36. The vector according to any of claims 32 to 35, wherein the origin of replication according to element (4) is selected from an origin which allows propagation of the vector is *S. cerevisiae*, preferably the two micron based or CEN/ARS origin of replication or more preferably an origin of replication that allows a high copy number in *S. cerevisiae*, preferably the two micron origin of replication.

37. A vector according to any of claims 32 to 36, wherein the promoter element according to element (5a) is selected from CYC1, GAL1, CUP1, ADH1 or TEF1.

38. A vector according to any of claims 32 to 37, wherein the open reading frame according to element (5b) encodes a part of the yeast ubiquitin comprising amino acids 1 to 37 of the wild-type or said part bearing an amino acid exchange at either position 3 or position 13 or both, wherein the amino acid replacing the original amino acid is preferably selected from leu, val, ala or gly.

39. A vector according to any of claims 32 to 38, wherein the tag is selected from the 3x FLAG epitope, 3x MYC epitope, HA epitopes or an hemagglutinin epitope.

40. The vector according to any of claims 32 to 39, wherein the site capable of receiving a further sequence according to element (5d) is selected from a multiple cloning site or a sequence allowing insertion of the further sequence by *in vivo* recombination.

41. A vector according to any of claims 32 to 40, wherein the terminator sequence is selected from yeast ADH1 or CYC1 terminators.

42. The vector according to any of claims 32 to 41, wherein the vector contains a further sequence encoding a protein or part thereof (the prey protein) which protein is either membrane bound or soluble.

43. Host cell containing the vectors and/or plasmids as defined in any of claims 9 to 42.

44. Use of the kit according to any of claims 9 to 17 or of the vector according to any of claims 18 to 41 in a process for detecting interaction between a first membrane bound protein or part thereof and a second protein or part thereof which is either membrane bound or soluble.

45. Use of the host cell according to claim 43 in a method for detecting an interaction between a first membrane bound protein or part thereof and a second protein or part thereof which is either membrane bound or soluble.

46. Use of the kit according to any of claims 9 to 17 or the vector according to any of claims 18 to 41 or the host cell of claim 43 in a screening process for identifying pharmaceutical drugs.

47. A method for providing a compound capable of interfering with protein/protein interaction comprising the following screening steps:

(1) providing a host cell according to claim 43, the bait and prey polypeptides being selected such that they interact when expressed in the host cell;
(2) incubating said host cell in the presence and absence of the compound(s) to be tested;
(3) measuring the difference in reporter gene expression between the incubation containing the compound(s) to be tested and the incubation free of the compound(s) to be tested; and optionally
(4) purifying or synthesizing the compound positively tested with the screen.